Molecular Basis of Cholera Toxin Action in Mammalian Cells: Modulation of Toxin ADP-ribosyltransferase Activity by Endogenous ADP-ribosylation Cycles

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Cholera toxin, produced by Vibrio cholerae, is responsible, in large part, for the devastating diarrhea characteristic of cholera. The toxin exerts its effects on cells through the ADP-ribosylation of Gαs, the stimulatory guanine nucleotide-binding protein that activates adenylyl cyclase, resulting in increased intracellular cyclic AMP content, and the often life-threatening derangements in fluid and electrolyte fluxes that are characteristic of the disease (1). Mammalian cells contain NAD:arginine ADP-ribosyltransferases, which, similar to cholera toxin, catalyze the modification of arginine residues in proteins; the ADP-ribose-arginine bonds are cleaved by ADP-ribose-(arginine)protein hydrolases (ARH), which regenerate the free (arginine)protein, thus completing an ADP-ribosylation cycle (2). We identified three genes that encode proteins with ARH-like sequences (3). Only one of the protein products of these genes, ARH1, was capable of cleaving ADP-ribose-(arginine)protein (3). We hypothesized that ARH1, if capable of cleaving ADP-ribose(arginine)Gαs, synthesized by cholera toxin, might reverse or limit the effects of cholera toxin on intestinal cells. In ARH1, aspartate residues 60 and 61 are critical for activity; their replacement with alanine results in an ARH1 protein with <0.1% of wild-type activity (4). ARH1 knockout mice were generated by replacing the exon containing the critical aspartate residues with a neomycin cassette. The ARH1 knockout mice are viable. Effects of cholera toxin on fluid accumulation in intestinal loops were significantly greater in the knockout than in the wild-type animals. Effects of cyclic AMP, the downstream signaling molecule generated by cholera toxin-catalyzed ADP-ribosylation were not, however, different in intestinal loops of the wild-type and knockout animals. Cholera toxin-generated ADP-ribosylarginine content and Gαs modification were significantly higher in intestinal epithelial cells from the knockout than from the wild-type mice. Similar findings were obtained with knockout and wild-type cells in culture. Thus, the effects of cholera toxin were significantly influenced by cellular ARH1 activity, which could function as a modifier gene in disease and serve as a potential therapeutic target. Presumably, in patients with cholera, toxin activity overwhelms this novel host defense, leading to persistent activation of adenylyl cyclase and the diarrheal syndrome characteristic of the disease.

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